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Low-Frequency Vibrational Spectra of Chlorophylls *a* and *b* in Solution: Effects of Axial Coordination

Masao Fujiwara*, Hidenori Hayashi, and Mitsuo Tasumi**

Department of Chemistry, Faculty of Science, The University of Tokyo, Bunkyo-ku,
Tokyo 113, Japan

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The far-infrared spectra (500—100 cm^{-1}) of chlorophyll *a* were observed in three solvent systems (benzene-pyridine, benzene-tetrahydrofuran, and benzene-acetone). The observed spectra could be classified into three types corresponding to the aggregate state, the five-coordinate monomeric state, and the six-coordinate state. The far-infrared spectra of ^{15}N - and ^{26}Mg -substituted species and the polarized resonance Raman spectra of chlorophyll *a* and pheophytin *a* in the region of 500—100 cm^{-1} were studied. These experimental results indicate that bands characteristic of the three states are due to modes to which deformations of the macrocycle make major contributions and vibrations around the Mg atom make some contributions, and that no band observed in the far-infrared and Raman spectra is attributable to a vibrational mode associated predominantly with the Mg atom and the coordinating atoms.

The spectral behavior of chlorophyll *b* in the benzene-pyridine system was similar to that of chlorophyll *a*.

INTRODUCTION

The coordination interaction of the Mg atom of chlorophylls with axial ligands is a matter of keen interest in photosynthesis.¹⁻³ The coordination number of the Mg atom is known to be usually five or sometimes six. The five-coordinate species has one axial ligand, and the six-coordinate species two axial ligands. Equilibrium between these two species was first found from visible absorption studies in solution.^{4,5}

Later, Cotton and Van Duyne⁶ investigated the coordination state of bacteriochlorophyll *a* *in vitro* by resonance Raman spectroscopy. In our recent paper,⁷ the effects of axial coordination to chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) in various solvents were studied. In these vibrational studies, it was shown that a few bands in the 1620—1510 cm^{-1} region were sensitive to the coordination number of the Mg atom. The bands in this spectral region mainly arise from the C=C stretches. These bands show downshifts when the chlorin ring expands on six coordination.

* Present address: Department of Chemistry, Faculty of Science, Hiroshima University, Naka-ku, Hiroshima 730, Japan.

** Author to whom correspondence should be addressed.

On the other hand, it is expected that bands in the far-infrared region would also be affected by the interaction between the Mg atom and the axial ligands. To test the validity of this expectation, the far-infrared spectra (500–100 cm^{-1}) of Chl *a* were compared with the mid-infrared ones (1800–1450 cm^{-1}).

Far-infrared measurements were made for many solution samples of Chl *a*, Chl *b*, and pheophytin *a*, in which the mixing ratios of two kinds of solvents were varied. Successful measurements could be carried out relatively easily, thanks to the recent advent of Fourier-transform infrared spectrophotometers. Thus, it may be worth noting that Fourier-transform far-infrared spectroscopy now provides a practical tool for studying intermolecular interactions in solution for a wide range of molecules.

The isotope shifts of Chl *a* were examined by substituting the Mg and N atoms of Chl *a* with ^{26}Mg and ^{15}N .

Raman measurements were also made to supplement the data already reported by other authors.³

Effects of axial coordination on the far-infrared bands and characterization of these bands and the low-frequency Raman bands will be discussed.

EXPERIMENTAL

Chl *a* and Chl *b* were prepared in the same manner as described before.⁷ No particular drying procedure for chlorophylls was carried out. Isotopically labeled pigments were extracted with methanol from green algae *Scenedesmus obliquus* cultured in a medium⁸ containing $^{26}\text{MgSO}_4$ or K^{15}NO_3 (95.8%, Prochem). For this purpose, 8 mg ^{26}MgO (97.1%, CEA) was dissolved in a minimum volume (0.5 ml) of 20% H_2SO_4 , and the resultant solution was added to the medium (1 l). Pyridine- d_5 (99.5%) was purchased from CEA, and the other solvents were of reagent grade.

Mid- and far-infrared spectra were recorded on a JEOL JIR-100 Fourier-transform infrared spectrophotometer. A TGS detector was used in the mid-infrared region, and a DTGS detector in the far-infrared region. Sample solutions containing 0.10 M chlorophylls were placed between KBr plates (for mid-infrared) or held in a polyethylene cell of path length 1 mm (for far-infrared). Solvent absorptions were numerically subtracted from each spectrum. For measurements of isotope shifts, spectra were observed at a resolution of 2 cm^{-1} , and four-times zero filling was employed in the Fourier transform. The precision of the frequency observed by this spectrometer was better than 0.01 cm^{-1} .

Resonance Raman spectra were observed for solid chlorophylls at 77 K and for $1.0\text{--}4.0 \times 10^{-3}$ M solutions of chlorophylls at room temperature. An NEC GLG-3300 Ar⁺ laser (457.9 nm), an NEC GLG-2018 He-Cd laser (441.6 nm), a Spex 1401 double monochromator, and a Hamamatsu R649 photomultiplier were used. The laser power was 10–60 mW at the sample. Calibration of the observed frequencies was performed with the bands of CCl_4 .

RESULTS AND DISCUSSION

Infrared Spectra of Chlorophyll a in Neat Benzene and Pyridine and Their Mixtures

The mid-infrared spectra of Chl *a* (1800–1450 cm^{-1}) in neat benzene and pyridine and their various mixtures are shown in Figure 1. Chl *a* in neat benzene shows three bands at 1737 (strong), 1693 (strong), and 1654 (medium) cm^{-1} in the carbonyl stretching region. These bands are assigned, respectively, to the ester carbonyl groups of the side chains, the keto carbonyl group at C-9 position, and the »aggregation peak«.⁹ The band at

1654 cm^{-1} completely disappears in the spectrum containing 0.77 mol% pyridine.

The spectrum in neat benzene shows three bands at 1609 (medium), 1553 (medium), and 1535 (medium) cm^{-1} in the C=C stretching region. The band at 1491 (medium) cm^{-1} is partly overlapped by a strong solvent absorption. As discussed previously,⁷ the band at 1609 cm^{-1} is coordination-sensitive. When the pyridine concentration exceeds 2.2 mol%, a new band at 1595 cm^{-1} appears. With increasing pyridine concentration, the intensity of the 1609 cm^{-1} band decreases and that of the 1595 cm^{-1} band increases. The former band is finally replaced by the latter in the spectrum containing 18 mol% pyridine. Such a downshift of the 1609 cm^{-1} band indicates that Chl *a*

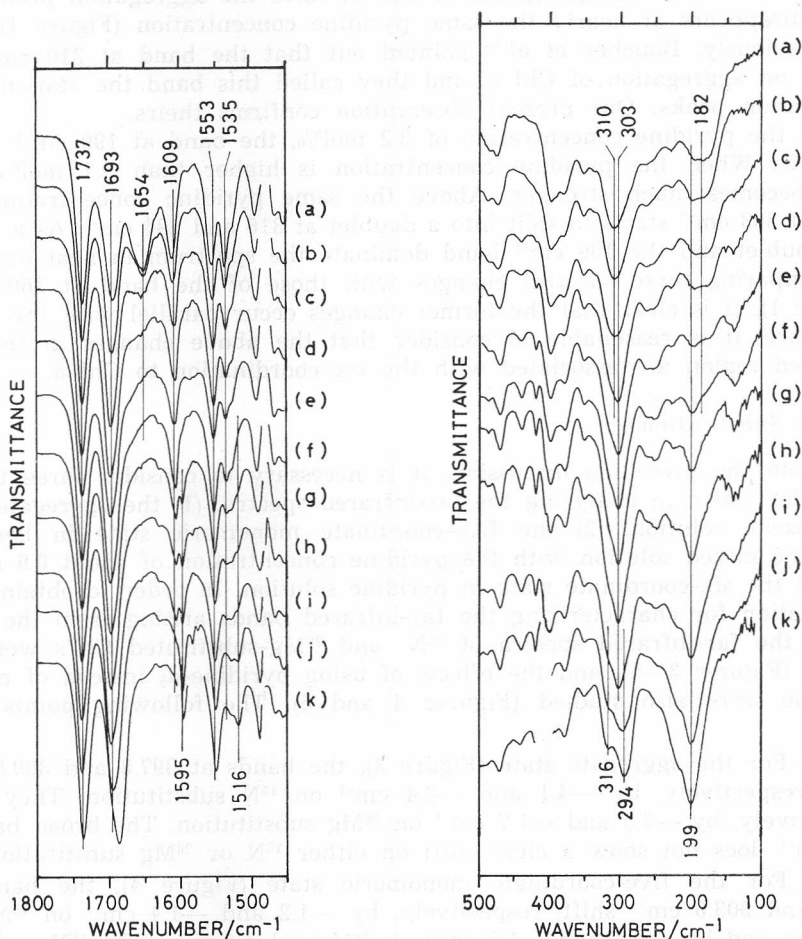


Figure 1 (left). Mid-infrared spectra of Chl *a* (0.10 M) in benzene-pyridine solutions. Pyridine concentration (mol%): (a) 0 (neat benzene); (b) 0.11; (c) 0.22; (d) 0.55; (e) 0.77; (f) 1.6; (g) 2.2; (h) 3.2; (i) 5.2; (j) 18; (k) 100 (neat pyridine).

Figure 2 (right). Far-infrared spectra of Chl *a* (0.10 M) in benzene-pyridine solutions. The measuring conditions for (a)-(k) are the same as those in Figure 1.

undergoes a change from the five-coordinate state to the six-coordinate. The band at 1553 cm^{-1} seems to be insensitive to aggregation and axial coordination to Chl *a*, and the band at 1535 cm^{-1} seems to be associated with both aggregation and axial coordination. The 1535 cm^{-1} band decreases in intensity with disaggregation and a new band at 1516 cm^{-1} appears upon six coordination.

The far-infrared spectra of Chl *a* ($500\text{--}100\text{ cm}^{-1}$) observed in benzene-pyridine mixtures are shown in Figure 2. In neat benzene Chl *a* has broad bands at 310 and 182 cm^{-1} . When a small amount of pyridine ($0.77\text{ mol}^0/\text{o}$) is added, the band at 310 cm^{-1} shifts to 303 cm^{-1} . At the same pyridine concentration, the band at 182 cm^{-1} weakens considerably. These changes must be associated with disaggregation of Chl *a*, since the aggregation peak (1654 cm^{-1}) disappears at nearly the same pyridine concentration (Figure 1).

Previously, Boucher *et al.*¹⁰ pointed out that the band at 310 cm^{-1} appeared on aggregation of Chl *a*, and they called this band the »far-infrared aggregation peak«. Our present observation confirms theirs.

At the pyridine concentration of $2.2\text{ mol}^0/\text{o}$, the band at 199 cm^{-1} begins to grow. When the pyridine concentration is higher than $5.2\text{ mol}^0/\text{o}$, this band becomes much stronger. Above the same pyridine concentration, the band at 303 cm^{-1} starts to split into a doublet at 316 and 294 cm^{-1} . As a result, this doublet and the 199 cm^{-1} band dominate the spectrum in neat pyridine. By comparing these spectral changes with those of the band at 1609 cm^{-1} (Figure 1), it is clear that the former changes occur parallel with the latter. Therefore, it is reasonable to consider that the above changes in the far-infrared region are associated with the six coordination to Chl *a*.

Isotope Substitution

From the preceding discussion, it is necessary to consider three typical states for Chl *a* in analyzing the far-infrared spectra: (1) the aggregate state in benzene solution, (2) the five-coordinate monomeric state in benzene-pyridine mixed solution with the pyridine concentration of about $0.9\text{ mol}^0/\text{o}$, and (3) the six-coordinate state in pyridine solution. In order to obtain more information for characterizing the far-infrared bands arising from the three states, the far-infrared spectra of ^{15}N - and ^{26}Mg -substituted Chl's were observed (Figures 3–5), and the effects of using pyridine- d_5 instead of normal pyridine were also studied (Figures 4 and 5). The following points were noted.

(1) For the aggregate state (Figure 3), the bands at 397.8 and 309.8 cm^{-1} shift, respectively, by -4.1 and -2.4 cm^{-1} on ^{15}N substitution. They shift, respectively, by -3.5 and -1.7 cm^{-1} on ^{26}Mg substitution. The broad band at 182 cm^{-1} does not show a clear shift on either ^{15}N or ^{26}Mg substitution.

(2) For the five-coordinate monomeric state (Figure 4), the bands at 399.7 and 303.0 cm^{-1} shift, respectively, by -1.2 and -1.4 cm^{-1} on ^{15}N substitution and by -0.7 and -1.9 cm^{-1} on ^{26}Mg substitution. The ^{15}N and ^{26}Mg shifts of the bands at 262 , 250 , 197 , 172 , and 137 cm^{-1} cannot be definitely determined. On deuteration of pyridine, the 419.2 cm^{-1} band disappears and a rather sharp band appears at 382.1 cm^{-1} on top of a broad band at 380 cm^{-1} (Figures 4a and 4b). This shows that the 419.2 cm^{-1} band is due to a mode of the bound pyridine, which shifts to 382.1 cm^{-1} on deuteration. In the

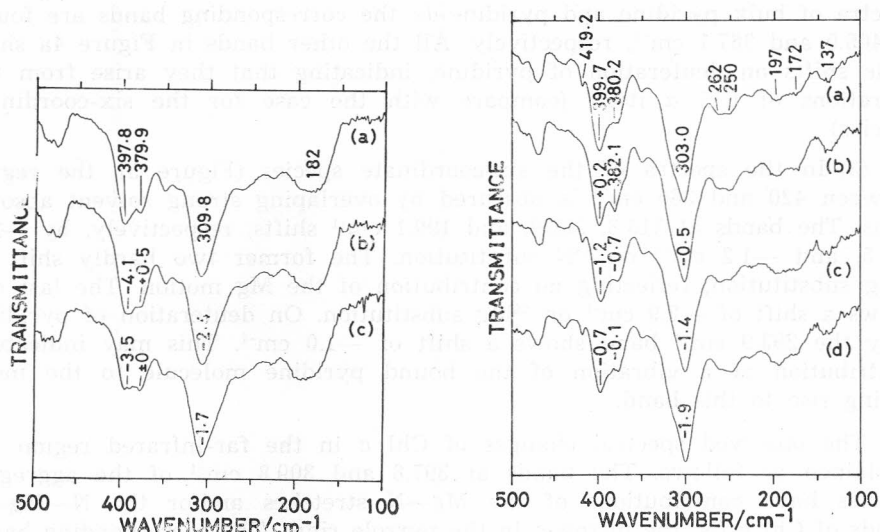


Figure 3 (left). Far-infrared spectra of Chl α (0.01 M) in benzene solution. (a) Native Chl α ; (b) ^{15}N -substituted Chl α ; (c) ^{26}Mg -substituted Chl α .

Figure 4 (right). Far-infrared spectra of Chl α (0.10 M) in benzene-pyridine solution (pyridine 0.90 mol%). (a) Native Chl α ; (b) native Chl α with pyridine- d_5 ; (c) ^{15}N -substituted Chl α ; (d) ^{26}Mg -substituted Chl α .

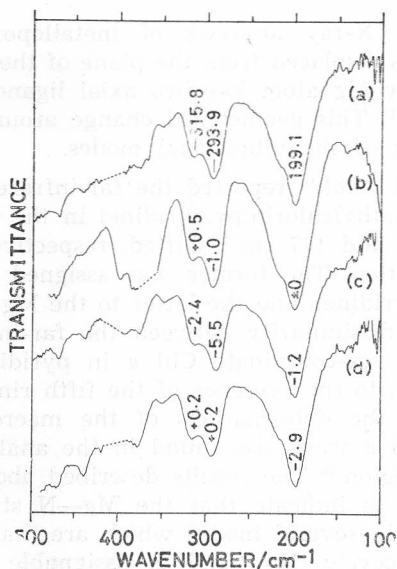


Figure 5. Far-infrared spectra of Chl α (0.10 M) in pyridine solution. (a) Native Chl α ; (b) native Chl α in pyridine- d_5 ; (c) ^{15}N -substituted Chl α ; (d) ^{26}Mg -substituted Chl α .

spectra of bulk pyridine and pyridine-*d*₅, the corresponding bands are found at 405.0 and 367.1 cm⁻¹, respectively. All the other bands in Figure 4a show little shifts on deuteration of pyridine, indicating that they arise from the vibrations of Chl *a* itself (compare with the case for the six-coordinate species).

(3) In the spectra of the six-coordinate species (Figure 5), the region between 420 and 360 cm⁻¹ is obscured by overlapping strong solvent absorptions. The bands at 315.8, 293.9, and 199.1 cm⁻¹ shifts, respectively, by -2.4, -5.5, and -1.2 cm⁻¹ on ¹⁵N substitution. The former two hardly shift on ²⁶Mg substitution, reflecting no contribution of the Mg motion. The last one shows a shift of -2.9 cm⁻¹ on ²⁶Mg substitution. On deuteration of pyridine, only the 293.9 cm⁻¹ band shows a shift of -1.0 cm⁻¹. This may indicate a contribution of a vibration of the bound pyridine molecule to the mode giving rise to this band.

The observed spectral changes of Chl *a* in the far-infrared region are explained as follows. The bands at 397.8 and 309.8 cm⁻¹ of the aggregate species have contributions of the Mg—N stretches and/or the N—Mg—N bends of Chl *a* (N, the nitrogen in the pyrrole ring). The corresponding bands of the five-coordinate monomeric and six-coordinate species may be assigned to modes to which the Mg—N stretches and/or the N—Mg—N bends contribute, but not in exactly the same manner as in the aggregate species. As a result of such mode rearrangements, the vibrational frequencies, intensities, and isotope shifts of the far-infrared bands depend upon the state of Chl *a*. The 199 cm⁻¹ band of the six-coordinate species is also assigned to a mode to which the Mg—N stretches and/or the N—Mg—N bends make some contributions.

According to the X-ray analyses of metalloporphyrins,¹¹⁻¹³ the five-coordinate Mg atom is displaced from the plane of the porphyrin ring toward the axial ligand. If the Mg atom has two axial ligands, it lies in the center of the porphyrin ring.¹⁴ This geometrical change around the Mg atom would induce rearrangements of the vibrational modes.

Previously, Ogoshi *et al.*¹⁵ reported the far-infrared spectra of Mg(OEC)-(py)₂ (OEC, *trans*-octaethylchlorin;py,pyridine) in the solid state. They found that the bands at 228 and 177 cm⁻¹ shifted, respectively, by -5.5 and -4.0 cm⁻¹ on ²⁶Mg substitution. The former was assigned to the Mg—N* stretch (N*, the nitrogen of pyridine), and the latter to the Mg—N (OEC) stretch. Rather surprising is the dissimilarity between the far-infrared spectra of solid Mg(OEC)(py)₂ and the six-coordinate Chl *a* in pyridine solution. This must be due, at least partly, to the presence of the fifth ring in Chl *a* which gives significant effects on the deformations of the macrocycle. The differences between OEC and Chl *a* were also found in the analysis of the band series in the mid-infrared region.¹⁶ The results described above for the far-infrared bands of Chl *a* seem to indicate that the Mg—N stretches and N—Mg—N bends are distributed in several modes which are mainly regarded as deformations of the macrocycle. No band is assignable to a mode consisting primarily of the Mg—N stretches and/or N—Mg—N bends. As discussed later, the N*—Mg—N* antisymmetric stretch is not observed in the far-infrared spectrum of Chl *a* in pyridine solution.

Far-infrared Spectra of Chl a in Other Solvents

Spectral changes of Chl *a* in the far-infrared region in different states are considered to originate mainly from rearrangements of the vibrational modes of this molecule. Then, a question arises as to whether the same effects occur on the spectra observed in other solvents which can be axial ligands. To check this point, the far-infrared spectra of Chl *a* in benzene-tetrahydrofuran (THF) and benzene-acetone solutions were observed (Figures 6 and 7).

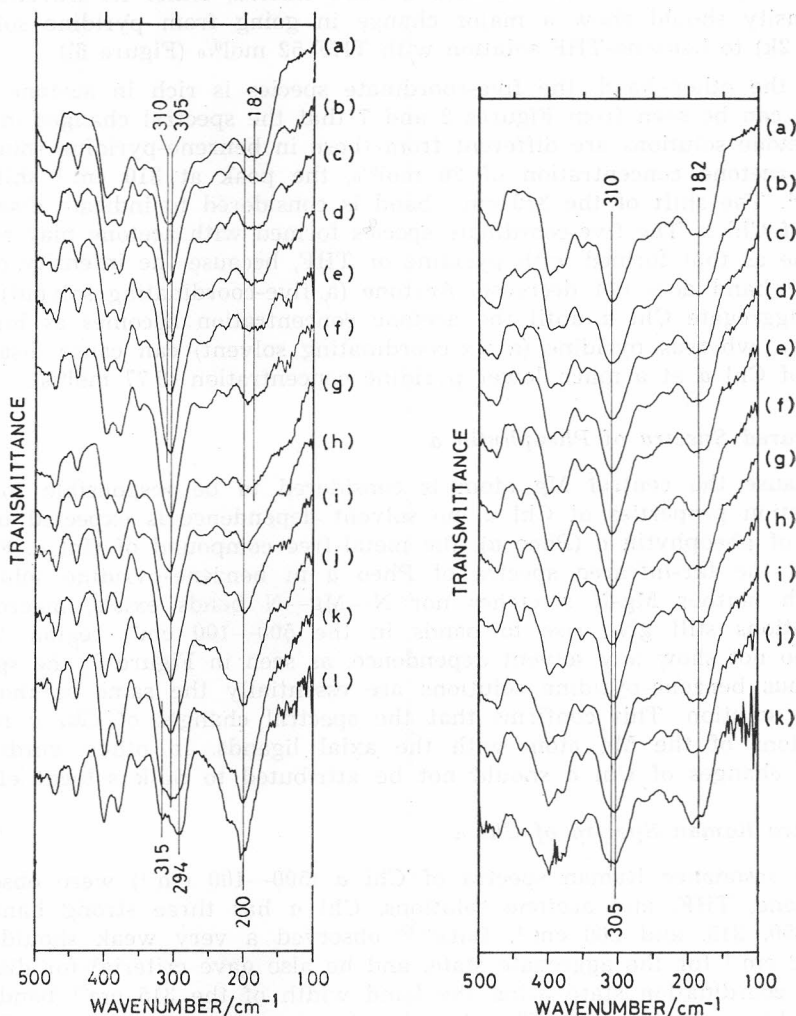


Figure 6 (left). Far-infrared spectra of Chl *a* (0.10 M) in benzene-tetrahydrofuran (THF) solutions. THF concentration (mol%): (a) 0 (neat benzene); (b) 0.11; (c) 0.22; (d) 0.55; (e) 0.77; (f) 1.6; (g) 2.2; (h) 3.2; (i) 5.2; (j) 9.9; (k) 18; (l) 52.

Figure 7 (right). Far-infrared spectra of Chl *a* (0.10 M) in benzene-acetone solutions. Acetone concentration (mol%): (a) 0 (neat benzene); (b) 0.24; (c) 0.60; (d) 0.84; (e) 1.8; (f) 2.4; (g) 3.5; (h) 5.7; (i) 11; (j) 20; (k) 55.

It is known that the six-coordinate species is dominant in THF solution.⁷ As seen in Figure 6, the spectral changes observed for various benzene-THF solutions of Chl *a* are similar to those for benzene-pyridine solutions (Figure 2). This implies that such changes are commonly observed when benzene solution of Chl *a* is titrated with other solvents which can form the six-coordinate species of Chl *a*. The above findings demonstrate that no band in the far-infrared spectra of Chl *a* is primarily associated with a mode which can be called the Mg-axial ligand stretch or axial ligand-Mg-axial ligand antisymmetric stretch. If such a band existed, either its wavenumber or intensity should show a major change in going from pyridine solution (Figure 2k) to benzene-THF solution with THF 52 mol% (Figure 6l).

On the other hand, the five-coordinate species is rich in acetone solution.⁷ It can be seen from Figures 2 and 7 that the spectral changes in benzene-acetone solutions are different from those in benzene-pyridine solutions. At the acetone concentration of 20 mol%, the peak at 310 cm⁻¹ shifts to 305 cm⁻¹. The shift of the 310 cm⁻¹ band is considered to indicate disaggregation of Chl *a*. The five-coordinate species formed with acetone may not be the same as that formed with pyridine or THF, because the intensity of the 182 cm⁻¹ band does not decrease. Acetone (a five-coordinating solvent) cannot disaggregate Chl *a* until the acetone concentration becomes as high as 20 mol%, whereas pyridine (a six-coordinating solvent) can cause disaggregation of Chl *a* at a much lower pyridine concentration (0.77 mol%).

Far-infrared Spectra of Pheophytin a

Because the central Mg atom is considered to be responsible for the coordination properties of Chl *a*, no solvent dependence is expected in the spectra of pheophytin *a* (Pheo *a*), the metal-free compound of Chl *a*. Figure 8 shows the far-infrared spectra of Pheo *a* in benzene-pyridine solutions. Although neither Mg-N stretches nor N-Mg-N bends exists, macrocycle deformations still give rise to bands in the 500–100 cm⁻¹ region. These bands do not show any solvent dependence, as seen in Figure 8; the spectra in various benzene-pyridine solutions are essentially the same as those in benzene solution. This confirms that the spectral changes of Chl *a* reflect interactions of the Mg atom with the axial ligands. In other words, the spectral changes of Chl *a* should not be attributed to bulk solvent effects.

Resonance Raman Spectra of Chl a

The resonance Raman spectra of Chl *a* (500–100 cm⁻¹) were observed in benzene, THF, and acetone solutions. Chl *a* has three strong bands at about 350, 315, and 260 cm⁻¹. Lutz^{17,18} observed a very weak shoulder at 312–302 cm⁻¹ for the aggregate state, and he also gave criteria³ for discerning the coordination state using the band width of the 315 cm⁻¹ band (320 cm⁻¹ in his measurements). The shoulder due to aggregation was confirmed in our measurements, but the criteria for the coordination state did not seem as obvious. We could not observe significant differences between the Raman spectra of Chl *a* in THF and acetone solutions. Thus, it is clear that the far-infrared spectrum gives better key bands for discerning the coordination state.

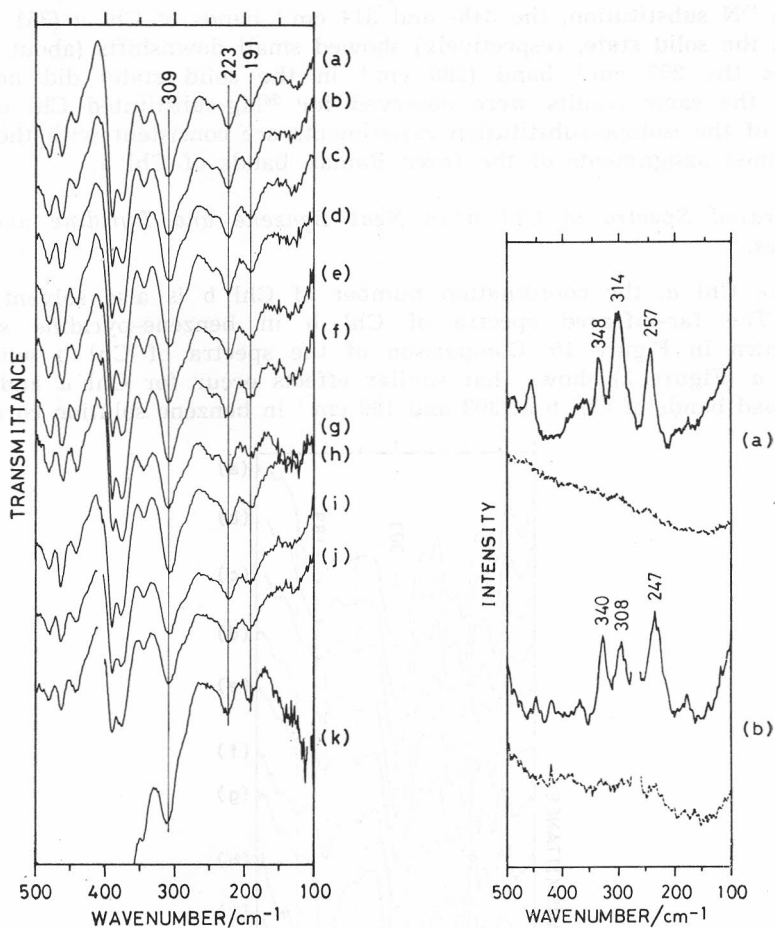


Figure 8 (left). Far-infrared spectra of pheophytin *a* (0.10 M) in benzene-pyridine solutions. The measuring conditions for (a)-(k) are the same as those in Figure 1.

Figure 9 (right). Polarized resonance Raman spectra of (a) Chl *a* (4.0×10^{-3} M) and (b) Pheo *a* (1.0×10^{-3} M) in tetrahydrofuran solution. Excitation wavelength: (a) 457.9 nm; (b) 441.6 nm. Full curve, polarized; broken curve, depolarized.

To characterize the low-frequency Raman bands of Chl *a*, we observed the polarized Raman spectra of Chl *a* and Pheo *a* in THF solution and the isotope shifts for Chl *a* in the solid state. As shown in Figure 9, the bands of Chl *a* at 438, 314, and 257 cm⁻¹ are polarized. They closely correspond, respectively, to the polarized bands of Pheo *a* at 340, 308, and 247 cm⁻¹, although the relative intensity of the 308 cm⁻¹ band of Pheo *a* decreases. Therefore, these bands should be attributed to nearly symmetric deformations of the macrocycle. The similarity between the spectra of Chl *a* and Pheo *a* indicates that the Mg—N stretches, N—Mg—N bends, and N*—Mg—N* symmetric stretch do not heavily contribute to the modes giving rise to the above three Raman bands of Chl *a*.

On ^{15}N substitution, the 348- and 314 cm^{-1} bands of Chl *a* (351 and 317 cm^{-1} in the solid state, respectively) showed small downshifts (about 1 cm^{-1}), whereas the 257 cm^{-1} band (260 cm^{-1} in the solid state) did not shift. Almost the same results were observed for ^{26}Mg -substituted Chl *a*. These results of the isotope-substitution experiments are consistent with the above-mentioned assignments of the three Raman bands of Chl *a*.

Far-infrared Spectra of Chl b in Neat Benzene and Pyridine and Their Mixtures.

Like Chl *a*, the coordination number of Chl *b* is also solvent dependent.⁷ The far-infrared spectra of Chl *b* in benzene-pyridine solutions are shown in Figure 10. Comparison of the spectra of Chl *b* with those of Chl *a* (Figure 2) shows that similar effects occur for Chl *a* and Chl *b*. The broad bands of Chl *b* at 303 and 189 cm^{-1} in benzene solution correspond,

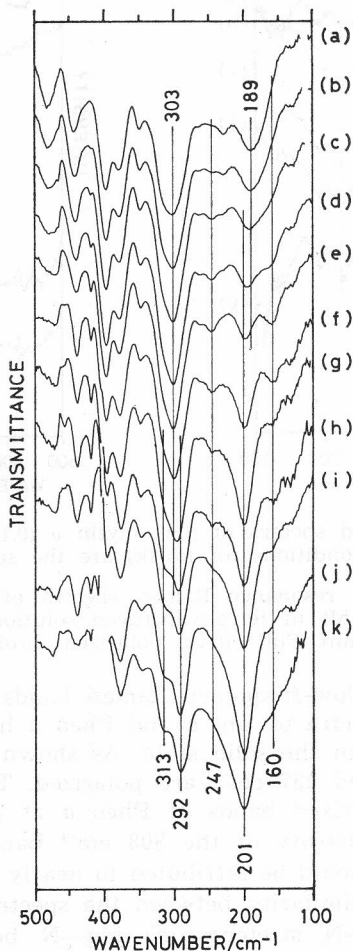


Figure 10. Far-infrared spectra of Chl *b* (0.10 M) in benzene-pyridine solutions. The measuring conditions for (a)-(k) are the same as those in Figure 1.

respectively, to those of Chl *a* at 310 and 182 cm^{-1} , indicating aggregation of Chl *b*. At the pyridine concentration of 0.77 mol%, the 303 cm^{-1} band sharpens, and the 189 cm^{-1} band loses its intensity, allowing the bands at 247, 201, and 160 cm^{-1} to appear. The sharpening of the 303 cm^{-1} band may be explained by assuming that a new sharp component appears at the same frequency. It is considered that Chl *b* is disaggregated at this pyridine concentration. In neat pyridine, the band at 303 cm^{-1} is split into a doublet at 313 and 292 cm^{-1} . The band at 201 cm^{-1} becomes much stronger. These features show that Chl *b* is six-coordinate in pyridine solution.

A difference between Chl *a* and Chl *b* may be that Chl *b* has a higher affinity to two axial ligands. The band characteristic of the six-coordinate species (at 201 cm^{-1}) begins to appear at lower pyridine concentration for Chl *b* (1.6 mol%) than for Chl *a* (2.2 mol%).

CONCLUSION

The far-infrared spectrum of Chl *a* has a few characteristic bands for the following three states of Chl *a*, *viz.*, the aggregate state, the five-coordinate monomeric state, and the six-coordinate state.

(1) The aggregate state has two characteristic bands at 310 and 182 cm^{-1} . Both of them are broad.

(2) The five-coordinate monomeric state shows a far-infrared spectrum similar to that of the aggregate state, but has a characteristic band at 305–303 cm^{-1} instead of the 310 cm^{-1} band of the aggregate state. The intensity of the 182 cm^{-1} band for the five-coordinate monomeric state varies with the kind of axial ligand.

(3) The six-coordinate state is characterized by a doublet at 316–315 and 294 cm^{-1} and a strong band at 200–199 cm^{-1} .

Chl *b* also shows similar spectral patterns characteristic of the above three states.

The far-infrared bands and the low-frequency Raman bands in the region of 500–100 cm^{-1} are due to modes to which deformations of the macrocycle make major contributions and vibrations around the Mg atom make some contributions. There is no band attributable to a vibrational mode associated predominantly with the Mg atom and the coordinating atoms [pyrrolic nitrogens and axial ligand(s)].

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SAŽETAK

Niskofrekvencijski vibracijski spektri klorofila *a* i *b* u otopini: Efekti aksijalne koordinacije

Masao Fujiwara, Hidenori Hayashi i Mitsuo Tasumi

Snimljeni su daleki infracrveni spektri ($500\text{--}100\text{ cm}^{-1}$) klorofila *a* u tri sustava otapala (benzen-piridin, benzen-tetrahidrofuran i benzen-aceton). Spektri se mogu klasificirati u tri tipa, koji odgovaraju agregiranom stanju, 5-koordiniranom monomernom stanju i 6-koordiniranom stanju. Proučeni su i daleki infracrveni spektri ^{15}N - i ^{26}Mg -supstituiranih vrsta, kao i polarizirani rezonantni Ramanovi spektri klorofila *a* i feofitina u području od $500\text{--}100\text{ cm}^{-1}$. Ovi eksperimentalni rezultati upućuju na to, da vrpce koje su karakteristične za tri navedena stanja potječu od vibracija s najvećim udjelom deformacije makrocikla i slabijim udjelom atoma Mg, dok u dalekim infracrvenim i Ramanovim spektrima nije otkrivena ni jedna vrpca koja bi se mogla pripisati vibraciji s dominantnim udjelom atoma Mg i koordiniranih atoma.

Spektralno ponašanje klorofila *b* u sustavu benzen-piridin slično je onome klorofila *a*.